STN/East

#13

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      (FILE 'HOME' ENTERED AT 08:35:58 ON 05 NOV 2002)

      FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:36:04 ON 05 NOV
      2002
L1         34735 S ALCOHOL (1N) DEHYDROGENASE
L2          4751 S L1 AND (MUTA? OR MODIFI?)
L3            32 S L2 AND (ACIDIC)
L4             3 S L3 AND NADH
L5             3 DUP REM L4 (0 DUPLICATES REMOVED)

      FILE 'STNGUIDE' ENTERED AT 08:37:43 ON 05 NOV 2002

      FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:38:01 ON 05 NOV
      2002
L6             7 S L3 AND INCREAS?
L7             4 DUP REM L6 (3 DUPLICATES REMOVED)
L8             4 S L3 AND ASPARTATE
L9             4 DUP REM L8 (0 DUPLICATES REMOVED)

=> s l1 and brevis
L10           31 L1 AND BREVIS

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11           26 DUP REM L10 (5 DUPLICATES REMOVED)

=> s l11 and (muta? or modif?)
L12            2 L11 AND (MUTA? OR MODIF?)
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(FILE 'HOME' ENTERED AT 09:06:01 ON 05 NOV 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 09:06:05 ON 05 NOV 2002

| | | | |
|---|---|---|---|
| L1 | 0 | S | BACTOBACILLUS (1N) BREVIS |
| L2 | 1646 | S | LACTOBACILLUS (1N) BREVIS |
| L3 | 29 | S | L2 AND (ALCOHOL (1N) DEHYDROGENASE) |
| L4 | 24 | | DUP REM L3 (5 DUPLICATES REMOVED) |
| L5 | 2 | S | L4 AND DNA |

| | Type | Hits | Search Text | DBs |
|---|---|---|---|---|
| 1 | BRS | 5229 | alcohol near1 dehydrogenase | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 2 | BRS | 94 | brevis and (alcohol near1 dehydrogenase) | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 3 | BRS | 68 | (brevis and (alcohol near1 dehydrogenase)) and mutant | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 4 | BRS | 21 | ((brevis and (alcohol near1 dehydrogenase)) and mutant) and aspartate | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 5 | BRS | 24 | ((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 6 | BRS | 24 | (((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and mutant | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 7 | BRS | 20 | ((((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and mutant) and aspartic | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 8 | BRS | 24 | (((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and brevis | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 9 | BRS | 8 | ((((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and brevis) and acidic | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 10 | BRS | 0 | "79610984" | USPAT; US-PGPUB; EPO; JPO; DERWENT; |
| 11 | BRS | 10 | "796914" | USPAT; US-PGPUB; EPO; JPO; DERWENT; |

L5 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1997:667746 CAPLUS
DN 127:289865
TI **Alcohol dehydrogenase** of Lactobacillus and its use in the enzymic production of chiral alcohols
IN Hummel, Werner; Riebel, Bettina
PA Boehringer Mannheim Gmbh, Germany
SO Eur. Pat. Appl., 34 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | EP 796914 | A2 | 19970924 | EP 1997-104814 | 19970320 |
| | EP 796914 | A3 | 19971210 | | |
| | R: CH, DE, ES, FR, GB, IT, LI | | | | |
| | DE 19610984 | A1 | 19970925 | DE 1996-19610984 | 19960321 |
| | JP 10028590 | A2 | 19980203 | JP 1997-87644 | 19970321 |
| | US 6037158 | A | 20000314 | US 1997-822322 | 19970321 |
| | US 6225099 | B1 | 20010501 | US 1999-466109 | 19991217 |
| PRAI | DE 1996-19610984 | A | 19960321 | | |
| | US 1997-822322 | A3 | 19970321 | | |

OS MARPAT 127:289865
AB An **alc. dehydrogenase** of Lactobacillus that is useful for the prepn. of chiral alcs. from ketones is described. The enzyme purified from **Lactobacillus brevis** had two pH optima (5.5 and 9.0) and a temp. optimum of 50.degree. and a very broad substrate specificity. The gene for the enzyme was cloned by PCR using sequence-derived probes and expressed in Escherichia coli using the com. expression vector pKK177.

L7 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
AN 1993:443992 CAPLUS
DN 119:43992
TI Substitution of Asp-223 residue to Leu in yeast **alcohol dehydrogenase** and coenzyme specificity
AU Lee, Kang Man; Ryu, Ji Won
CS Coll. Pharm., Ewha Womans Univ., Seoul, 120-750, S. Korea
SO Yakhak Hoechi (1992), 36(5), 469-73
CODEN: YAHOA3; ISSN: 0513-4234
DT Journal
LA Korean
AB Yeast **alc. dehydrogenase** (YADH) has an **acidic** residue that interacts with the 2'- and 3'-OH groups of the adenosine ribose moiety of NAD. The **acidic** residue, Asp-223 (according to the horse liver **alc. dehydrogenase** amino acid sequence), is supposed to det. the coenzyme specificity for NAD rather than NADP. Here, Asp-223 was replaced by leucine and the **mutant** YADH was expressed in yeast and characterized for the coenzyme specificity. The turnover nos. of the **mutant** enzyme for NAD and EtOH were decreased 3.5- and 4.8-fold compared to wild-type enzyme, resp. In contrast, the specificity for NADP was **increased** 13-fold. As a result, the **mutant** YADH was able to also employ NADP as a coenzyme.

L7 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2002 ACS
AN 1991:424940 CAPLUS
DN 115:24940
TI An aspartate residue in yeast **alcohol dehydrogenase** I determines the specificity for coenzyme
AU Fan, Fan; Lorenzen, James A.; Plapp, Bryce V.
CS Dep. Biochem., Univ. Iowa, Iowa City, IA, 52242, USA
SO Biochemistry (1991), 30(26), 6397-401
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
AB In the 3-dimensional structures of enzymes that bind NAD or FAD, there is an **acidic** residue that interacts with the 2'- and 3'-OH groups of the adenosine ribose moiety of the coenzyme. The size and charge of the carboxylate may repel the binding of the 2'-phosphate group of NADP and explain the specificity for NAD. In the NAD-dependent **alc. dehydrogenases**, Asp-223 (horse liver **alc. dehydrogenase** sequence) appears to have this role. The homologous residue in yeast **alc. dehydrogenase** I (residue 201 in the protein sequence) was substituted with glycine, and the D223G enzyme was expressed in yeast, purified, and characterized. The wild-type enzyme was specific for NAD. In contrast, the D223G enzyme bound and reduced NAD and NADP equally well, but, relative to wild-type enzyme, the dissocn. const. for NAD was **increased** 17-fold, and the reactivity (Vmax/Km) on EtOH was decreases to 1%. Even though the catalytic efficiency was reduced, yeast expressing the altered or wild-type enzyme grew at comparable rates, suggesting that equilibration of NAD and NADP pools is not lethal. Thus, Asp-223 participates in binding NAD and in excluding NADP, but it is not the only residue important for detg. specificity for coenzyme.

L12 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 2002:87234 CAPLUS
DN 136:130770
TI Dehydrogenase **mutants** capable of using NAD as coenzyme and their preparation and use for chiral hydroxy compound preparation
IN Riebel, Bettina; Hummel, Werner; Bommarius, Andreas
PA Degussa Aktiengesellschaft, Germany
SO Eur. Pat. Appl., 23 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | EP 1176203 | A1 | 20020130 | EP 2001-114953 | 20010620 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| | DE 10037101 | A1 | 20020207 | DE 2000-10037101 | 20000727 |
| PRAI | DE 2000-10037101 | A | 20000727 | | |

AB The NADH specificity of preferred NADPH-dependent dehydrogenases can be improved by reducing the basicity of the coenzyme binding site through genetic engineering. Dehydrogenases with NADH-dependence suitable for preparative purposes having a kcat/KM value for NAD+ .gtoreq.20 can be obtained with recombinant microorganisms. Thus, the enzyme gene is altered so that basic amino acid(s) in the coenzyme binding site are at least partially replaced by uncharged or neg. charged amino acids. The inventive method is esp. useful for obtaining short-chain dehydrogenases with coenzyme binding sites at the N-terminus. Thus, **mutant** Lactobacillus **brevis alc. dehydrogenases** were prepd. with recombinant Escherichia coli. The **mutant** contg. an aspartic acid at amino acid 38, rather than glycine, showed a 10-fold preference for NAD over NADP.
RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1999:614147 CAPLUS
DN 131:239737
TI Dehydrogenase **mutants** with improved NAD-dependence, their manufacture with recombinant microorganisms, and their use for chiral hydroxy compound preparation
IN Hummel, Werner; Riebel, Bettina
PA Forschungszentrum Julich G.m.b.H., Germany
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 9947684 | A2 | 19990923 | WO 1999-DE848 | 19990318 |
| | WO 9947684 | A3 | 20000323 | | |
| | W: JP, US | | | | |
| | RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| | DE 19812004 | A1 | 19990930 | DE 1998-19812004 | 19980319 |
| | EP 1002097 | A2 | 20000524 | EP 1999-923384 | 19990318 |
| | R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE | | | | |
| | JP 2001526547 | T2 | 20011218 | JP 1999-546407 | 19990318 |
| | US 6413750 | B1 | 20020702 | US 1999-447125 | 19991118 |
| PRAI | DE 1998-19812004 | A | 19980319 | | |
| | WO 1999-DE848 | W | 19990318 | | |

AB The NADH specificity of preferred NADPH-dependent dehydrogenases can be

improved by reducing the basicity of the coenzyme binding site through genetic engineering. Dehydrogenases with NADH-dependence suitable for preparative purposes having a kcat/KM value for NAD+ .gtoreq.20 can be obtained with recombinant microorganisms. Thus, the enzyme gene is altered so that basic amino acid(s) in the coenzyme binding site are at least partially replaced by uncharged or neg. charged amino acids. The inventive method is esp. useful for obtaining short-chain dehydrogenases with coenzyme binding sites at the N-terminus. Thus, **mutant** Lactobacillus **brevis alc. dehydrogenases** were prepd. with recombinant Escherichia coli. The **mutant** contg. A9G, G37D, R38L, and K48M substitutions used only NAD as coenzyme..